

## **PHYTOCHEMICAL SCREENING AND PHARMACOLOGICAL EVALUATION OF ANTICANCER ACTIVITY OF METHANOLIC EXTRACT OF MAYTENUS EMARGINATA (WILLD) IN RATS**

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### **ABSTRACT**

Cancer is one of the most serious health problems that affect the duration and quality of the individual's life. Enormous efforts are invested to cope with this problem, but unfortunately limited success has ever been achieved with most of the therapeutic strategies. These efforts are usually complicated with the need for well experienced surgeons, lack of specificity and high cost, as well as being usually accompanied with a wide range of side effects. As the conventional therapeutic strategies fail to fulfill the major requirements for a successful cancer therapy, the use of naturally developed anticancer agents has evolved as an alternative safe, low-cost and convenient one. Therefore, the use of plant extracts with potential anticancer

therapeutic effects might be particularly significant, especially in Palestine, which is rich in thousands of plant species known for their medical uses. Moreover, the lack of expertise, the scares economical resources and the complicated political situation in Palestine don't allow the application of sophisticated surgical, chemo- and radio-therapies to cure cancer. Therefore, the current study, investigates the effect of crude methanolic extracts from Maytenus emarginata, Fig on cell lines derived from different human tissue origins (Hep3b: Hepatocellular carcinoma; Hela: cervical epithelial cancer; and A549: human lung adrenal cancer). The results showed a concentration-dependent reduction in the final number of cancer cells in consequence to treatment with the aforementioned methanolic extracts. Two kinds of anticancer effects were evaluated and found to contribute to this reduction: the antiproliferation effect (decreased number of metabolically active cells) and cytotoxicity (decreased number of live cells). This extracts possess both of the effects with various

degrees. *Maytenus emarginata* possess the strongest and most profound effects on the three cell lines, mainly by induction of cell death. Further studies are needed to assess the active ingredients of *Maytenus emarginata*, involved in the antiproliferative or cytotoxic effects of these plants. These studies must involve the establishment of *in vivo* animal models and the application of more efficient extraction and fractionation techniques.

**KEYWORDS:** Anti-cancer, *Maytenus Emarginata*, In-vitro, Phytochemistry, Pharmacological Evaluation.

### Aim and Objectives

Pharmacological evaluation of methanolic extracts of *Maytenus emarginata* Fruits were taken. In the present work an attempt was done for evaluation of anti-cancer activity.

- To prepare methanolic extracts of *Maytenus emarginata*.
- To determine pharmacognostic studies.
- To evaluate the physicochemical parameters.
- To perform acute toxicity of these extracts.
- To determine the anti-cancer activity by using different cell-lines in rats.
- Cancer cell lines a549 (human lung adenocarcinoma epithelial cell line) and
- Hela (human cervical carcinoma cell line).

### INTRODUCTION

➤ The Indian subcontinent is a vast repository of medicinal plants that are used in traditional medical treatments.<sup>[1]</sup> Many westerners have long regarded the Indian systems of medicine as a rich source of knowledge. In India, around 20,000 medicinal plants have been recorded however traditional communities are using only 7,000 - 7,500 plants for curing different diseases.<sup>[2]</sup> Even today, majority of the medicines are prepared from the plant and animal products, minerals and metals etc. Major pharmaceutical industries depend on the plant products for the preparation of Ayurvedic medicines.

➤ Plants, especially used in Ayurveda can provide biologically active molecules and lead structures for the development of modified derivatives with enhanced activity and /or reduced toxicity. The small fraction of flowering plants that have so far been investigated have yielded about 120 therapeutic agents of known structure from about 90 species of plants. Some of the useful plant drugs include vinblastine, vincristine, taxol, podophyllotoxin, camptothecin, digitoxigenin, gitoxigenin, digoxigenin, tubocurarine, morphine, codeine,

aspirin, atropine, pilocarpine, capscicine, allicin, curcumin, artemesinin and ephedrine.<sup>[3]</sup> In some cases, the crude extract of medicinal plants may be used as medicaments. On the other hand, the isolation and identification of the active principles and elucidation of the mechanism of action of a drug is of paramount importance. Hence, work in both mixture of traditional medicine and single active compounds are very important.

## PHARMACOGNOSY

The word “pharmacognosy” was coined in the early 19th century to designate the discipline related to the study of medicinal plants.<sup>[4]</sup> The science of pharmacognosy became aligned with botany and plant chemistry, and until the early 20th century, dealt mostly with physical description and identification of whole and powdered plant drugs including their history, commerce, collection, preparation, and storage. Advances in organic chemistry added a new dimension to the description and quality control of these drugs, and the discipline has since expanded to include discovery of novel chemical therapeutic agents from the natural world.<sup>[5]</sup> Pharmacognosy studies help in identification and authentication of the plant material.

The process of standardization can be achieved by stepwise pharmacognostic studies.<sup>[6]</sup> The standardization of a crude drug is integral part of establishing its correct identity. Before any crude drug is included in herbal pharmacopoeia, pharmacognostic as well as other standard parameters must be established.<sup>[7]</sup> Therapeutic efficacies of medicinal plants depend upon the quality and quantity of chemical constituents. It has been established that chemical constituents of a plant species vary with regard to climate and seasons.<sup>[8]</sup> A number of different bases are used for morphological studies and a natural variation in these characteristics play an important role for preliminary evaluation of crude drugs. The basis of analysis by evaluation of microscopic characters is that there are always sufficient differences in the same type or different types of plants as far as the cell characteristics are concerned. Standardization profiles of herbal drugs are not available for most drugs.<sup>[9]</sup> The therapeutic activity of herbs is because of various constituents present in them. Therapeutic efficacy of medicinal plants depends upon the quality and quantity of chemical constituents which may vary depending on various factors, one amongst is the geographical localities which show quantitative variation in their chemical constituents. In some plants toxic constituents are also present therefore it is essential to evaluate their quality, safety and efficacy. Correct identification and quality assurance of the starting material is therefore an essential prerequisite to ensure producible quality of herbal medicine, which contributes to its safety

and efficacy.<sup>[10&11]</sup> In most of the cases of herbal medicine, misuse starts with wrong identification. Many of the traditional systems have records where one common vernacular name is given to two or more entirely different species.<sup>[12]</sup>

**Macro and microscopic examination:** For identification of right variety and search of adulterants.

**Foreign organic matter:** Remove matter other than source plant to get the drug in pure form.

**Ash values:** It is criteria to judge the identity and purity of crude drug - Total ash, sulfated ash, water soluble ash, acid insoluble ash, etc.

**Moisture content:** Checking moisture content helps reduce errors in the estimation of the actual weight of drug material. Low moisture content suggests better stability against degradation of product.

**Extractive values:** These are indicative weights of the extractable chemical constituents of crude drug under in different solvents.

**Crude fiber:** This helps to determine the woody material component and it is a criterion for judging purity.

**Qualitative chemical evaluation:** It helps in identification and characterization of crude drug with respect to phytochemicals constituent. It employs different analytical techniques to detect and isolate the active constituents. Phytochemical screening techniques involve botanical identification, extraction with suitable solvents, purification and characterization of the active constituents of pharmaceutical importance.

**Chromatographic examination:** It involves identification of specific chemical constituents of crude drugs responsible for a specific activity and can be used as markers.

**Quantitative chemical evaluation:** To estimate the exact amount of phytoconstituents present in the crude drugs.

➤ **Toxicological studies:** Pesticide residue, potentially toxic elements, and microbial count which may reduce the efficacy of the final product.

**CANCER** is one of the most serious health problems worldwide, affecting individuals from different sexes, ages, and races. It is a group of diseases, characterized by uncontrolled cellular growth with frequent cancer cells invasion to different body parts and spreading to other organs, a process referred to as Metastasis. Metastasis is the major cause of cancer related mortality.<sup>[13]</sup> In 2005, cancer was the second leading cause of death among both men and women and accounted for 13% of the total 58 million deaths worldwide.<sup>[13]</sup> In 2006, about 10.9 million new cancer cases are expected to be diagnosed worldwide and more than 7.8 million cancer patients may die.<sup>[13]</sup> According to the latest report of cancer registry unit in Gaza strip, 5500 cases have been reported over the period from January, 1995 to December, 2003.<sup>[14]</sup> In addition, 1026 cancer patients died in 2004 in the Palestinian territories with a mortality rate of 28.2 per 100.000.<sup>[14]</sup>

➤ Cancer is also a problem of economical dimensions with a very high level of expenses associated to it. For example the National Institute of Health, USA estimates that an overall of \$209.9 billion were invested worldwide in 2005, for the sake of cancer research and management.<sup>[15]</sup> Cancer is a heterogeneous illness which can originate from many different organs of the human body. However, the most frequent cancer types in the world are lung, prostate, stomach, colorectal, and esophagus in men; and breast, lung, stomach, colorectal and cervical in women.

- Cancer (medical term: malignant neoplasm) is a class of diseases in which
  - · Group of cells display uncontrolled growth (division beyond the normal limits).
  - · Invasion (intrusion on and destruction of adjacent tissues).
  - · Sometimes metastasis (spread to other locations in the body via lymph or blood).
  - Three malignant properties of cancers differentiate them from benign tumors, which are self limited, and do not invade or metastasize.
1. Uncontrolled growth (division beyond the normal limit).
  2. Invasion (intrusion on and destruction of adjacent tissues).
  3. Metastasis (spread to other location in the body).

- Nearly all cancers are caused by abnormalities in the genetic material of the transformed cells. These abnormalities may be due to the effects of carcinogens, such as tobacco smoke, radiation, chemicals, or infectious agents. Other cancer-promoting genetic abnormalities may be randomly acquired through errors in DNA replication, or are inherited.

## MATERIALS AND METHODS

### PHARMACOGNOSTIC STUDIES

#### Macroscopic characteristics

The diagnostic characters of *M.emarginata* of fruit are the presence of quadrangular stem with winged corners and the internodes on four sides are invaded or depressed deeply in the middle and the corners are exerted with sharp reddish brown to black colored margins, 3-4 cm long (Fig. 2).



**Fig 2 Macroscopy of *M.emarginata***

#### Microscopic characteristics

Diagrammatic TS of the stem is four angled; on maturation each goes deep inside forming sharp pointed like projection and shows single layer epidermis followed by hypodermis; narrow cortex and centrally located large pith occupying almost  $2/3^{\text{rd}}$  region of the section, surrounded by numerous, small, discontinuous band of vascular bundles. Detailed section shows rectangular - pentagonal, 1-2 layered epidermis covered by thin cuticle, followed by 3-4 layered, circular-polygonal, chlorenchymatous hypodermis deposited more near the angle; cortex very narrow, cortical parenchymatous, 5-7 layered; pith very large, parenchymatous similar to that of region surrounded by discontinuous band of numerous, small, conjoint, collateral vascular bundles, each shielded with sclerenchymatous sheath, stele near the angle formed into strip, capped with collenchymatous band; few starch grains and rosette crystals and abundant large cells of mucilage, clusters and bundles of acicular crystals of calcium oxalate scattered throughout the section.

**Powder characteristic:** The fine powder is green in color with faint odor. The diagnostic features of powder are plenty of cluster, rosette and acicular crystals of calcium oxalate

scattered as such throughout or embedded in parenchymatous cells. Simple and compound starch grains 2-celled, scattered or embedded in parenchyma. Fragments of epidermis in surface view embedded with anisocytic stomata. The fibers are isolated or in groups, thin walled, occasionally exhibiting dentate margin, vessels with annular, reticulate and boarded pitted thickening. Cells of the medullary rays with pitted thickening.

### PHYSICOCHEMICAL ANALYSIS

The results of physicochemical analysis of crude powder of *M.emarginata* are shown in Table 2. The average values of various parameters are expressed as percentage of air-dried material. Loss on drying was 9.5 %. Total ash was 19.41 %, acid insoluble ash was 17.0 % and water soluble ash was 14.16 %. The extractive value of crude powder was maximum in water (19.18 %), followed by methanol (7.81 %) and minimum was in petroleum ether (1.11 %), pH of ME was 4.25.

### Solubility test

The ME of *M.emarginata* fruits was evaluated for its solubility in 11 solvents with varied polarities. The extract was highly soluble in dimethylformamide, distilled water and methanol but less soluble in ethyl acetate, 1-4 dioxan and petroleum ether (Table 3). Determination of solubility of ME of *M.emarginata* fruits in different solvents.

**Table 3: Physicochemical characteristics**

Sr. No.	Physicochemical parameters	Average value % W/W
1.	Loss on drying	09.50%
2.	Total ash	19.41 %
3.	Acid insoluble ash	17.00 %
4.	Water soluble ash	14.16 %
5.	Petroleum ether soluble extractive	01.11 %
6.	Ethyl acetate soluble extractive	02.08 %
7.	Acetone soluble extractive	01.94 %
8.	Methanol soluble extractive	07.81 %
9.	Water soluble extractive	19.18 %
10.	pH (ME)	04.23

### Phytochemical Analysis

Phytochemical analysis revealed the presence of secondary metabolites like alkaloids, flavonoids, cardiac glycosides and triterpenes (Table 4). However, the ME was rich in alkaloids (Wagner test) while crude powder was rich in cardiac glycosides.



**Table-4 Phytochemical analysis**

No.	Phytochemical	Test	Crude Powder	Methanol extract
1.	Alkaloids	Dragendroff test	+	+
		Mayer test	+	-
		Wagner test	+	+++
2.	Flavonoids	Shinoda test	-	-
		Alkaline reagent	+	-
3.	Cardiac Glycosides	Keller-kilianni test	+++	++
4.	Tanins	HCl test	-	-
		FeCl <sub>3</sub> test	-	-
5.	Saponins	Frothing test	-	+
6.	Steroids	Liebermann-Burchard reaction	+	+
7.	Triterpenes	H <sub>2</sub> SO <sub>4</sub> test	+	+

**Anticancer Activity****He La (Human cervical carcinoma) cell line**

The anticancer activity of ME of *M.emarginata* Fruits and its fractions against HeLa cell line was evaluated by MTT assay. Treatment with ME at concentration of 0.1, 1, 10 and 50 µg/ml for 24 h resulted in a concentration-dependent reduction in cell viability for HeLa cells. Estimated IC<sub>50</sub> value for suppression of cell proliferation at 24 h was 12.40 and 15.34µg/ml (Table 7). Treatment with standard doxorubicin at concentration for 24 h resulted in a concentration-dependent reduction in cell viability for He La cells.

**A549 (Human lung adenocarcinoma epithelial) cell line**

The anticancer activity of ME of *M.emarginata* Fruits and its fractions against A549 cell line was evaluated by MTT assay. Treatment with ME at concentration of 0.1, 1, 10 and 50 µg/ml for 24 h resulted in a concentration dependent reduction in cell viability for A549 cells. Estimated IC<sub>50</sub> value for suppression of cell proliferation at 24 h was 5.93 and 4.54µg/ml. (Table 5). Treatment with standard doxorubicin for 24 h resulted in a concentration dependent reduction in cell viability for A549 cells. Its IC<sub>50</sub> value was 0.376 µg/ml (Table 5). ME showed good anticancer activity against A549 cell line than the HeLa cell line. Effect on ME on the proliferation of HeLa and A549 cells was studied in both cell lines, number of cells in treated plate (ME) reduced than in the control plate.



**Table 5: The IC<sub>50</sub> value of anticancer activity of ME of *M.emarginata* Fruits**

Sr. No.	Name of Sample	IC <sub>50</sub> value (µg/ml)	
		He La	A549
1.	Control	20.5	9.54
2.	Doxorubicin	0.0701	0.376
3.	ME(400)	12.40	5.93
4.	ME(800)	15.34	4.54

## CONCLUSION

Maytenus Emarginata belongs to the family Celastraceae, an edible plant found in India. It is commonly known as “Tho My Soft Tree”. The fruits of Maytenus Emarginata are reported to have great medicinal value. Considering above, in the present work, anticancer and toxicity study was evaluated. In physicochemical analysis, the highest extractive value was obtained from water and methanol extract. The extract was maximum soluble in polar solvents like DMF, methanol and water; and was acidic in nature. In qualitative phytochemical analysis, cardiac glycosides and alkaloids were present in higher amount, while tannins were totally absent. The quantitative phytochemical investigation gave valuable information about the different phytoconstituents present in the powder extract, which helps the future investigators regarding the selection of the particular extract for further research in isolation of new active compounds. The total phenol content was higher than flavonoid content. Such Pharmacognostic study serves important criteria in standardization of the Maytenus Emarginata fruit, ensuring quality formulations. In anticancer studies, ME showed potent proliferation inhibitory activity against human lung adenocarcinoma epithelial cell line (A549) and human cervical carcinoma cell line (HeLa). This is the first report on the anticancer properties of Maytenus Emarginata. The ME showed good anticancer activity against A549 cell line than the HeLa cell line. In acute toxicity study, there is no mortality and observable acute toxic effect during the entire period in male and female rats dosed up to 800 mg/kg b.w. orally. Detailed experimental analysis on sub acute and chronic toxicity is essential for further support of this drug. These studies have shown that the ME of Maytenus Emarginata and its fractions contain some active ingredients with the potential of being good anticancer agents. Further work should be carried out on the characterization of specific antioxidant and anticancer components of Maytenus Emarginata and evaluation of their therapeutic significance in prevention of diseases induced by oxidative stress.

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